Regular Review

Radioimmunodetection of cancer: problems and potential

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Many patients and their clinicians would be grateful for a non-invasive method of detecting the extent of primary and secondary malignant tumours. The knowledge gained would enable treatment to be tailored to the exact needs of the individual patient. It would be ideal if the treatment could be delivered in such a way that only the areas affected by the tumour were treated. Ehrlich's dream of "magic bullets" may now be nearing reality thanks to the manufacture of tumour specific antibodies using the monoclonal antibody technique developed by Kohler and Milstein. Attempts to radiolabel the antibodies and detect their localisation in vivo—"radioimmunodetection"—have not, however, been an unqualified success.

Goldenberg's group in Kentucky have set the standard with their efforts to detect tumours using antibodies to carcinoembryonic antigen,^{1,2} α fetoprotein,³ and human chorionic gonadotrophin.⁴ A total of 142 patients were scanned at 24 and 48 hours with a gammacamera to detect the localisation of polyclonal goat anticarcinoembryonic antigen antibodies. They accurately located 85% of colorectal cancers, 88% of ovarian cancers, 90% of cervical cancers, 71% of lung cancers, 50% of pancreatic cancers, 40% of primary breast cancers, and 78% of metastatic breast cancers.² Plasma concentrations of carcinoembryonic antigen did not always correlate with the results of radioimmunodetection: tumour was seen in some cases with normal concentrations and missed in some with high concentrations. Most of the sites that were missed were less than 2 cm diameter, and some of the positive sites later confirmed as malignant were not identified with conventional detection methods.

Unfortunately, other groups have had difficulty in matching these results. Dykes *et al* were able to detect four of five primary sites and eight of 11 metastatic sites in 15 patients with gastrointestinal neoplasms using polyclonal sheep anticarcinoembryonic antigen antibodies.⁵ In three patients the technique was totally unsuccessful: in two cases technical problems may have contributed, but in the third there seems no explanation for the failure of the method to locate the tumour. Another study using goat and baboon anticarcinoembryonic antigen antibodies gave some disturbing results.⁶ Deiodination in vivo was rapid, up to half of the administered ¹³¹I being excreted in the urine within the first 24 hours, resulting in poor imaging. Four of 27 tumour sites were identified without resort to subtraction techniques, but false positive results were generated in every case with subtraction.

Subtraction techniques

Interpretation of the scans taken after injection of a radio-labelled antibody may be difficult because of background

radioactivity, which is due to the presence of labelled antibody outside the areas affected by tumour. The technique usually tried to circumvent this problem uses technetium labelled albumin and free technetium pertechnetate before the scanning procedure. Technetium is used to identify blood pool radioactivity and the non-specific accumulation of labelled protein in the stomach, bladder, and thyroid, and this image is subtracted, using a computer, from the image due to ¹³¹I, to give pictures identifying only tumour specific localisation. Unfortunately there are no objective criteria to determine the normal area or the degree of subtraction; so the method is not reproducible, and the images may be misinterpreted. Subtraction techniques may be improved, however, with the use of normal IgG instead of albumin and ¹²³I in place of ⁹⁹Tc as the radiolabel.

The characteristics of the radioisotope, the antibody, the tumour, and its antigens all contribute to the results of radiolocalisation. For instance, the binding of the isotope to the antibody should not significantly alter its affinity, specificity, or biological properties nor decrease the rate of clearance of the antibody from the body. The isotope must have the highest possible specific activity in order to maintain immune reactivity—which may drop by half after labelling.⁷ Iodination, for instance, impairs the biological properties of proteins.⁸

Several manoeuvres have been used to avoid these problems. Using a radioactive metal chelate as the label Rainsbury and Westwood obtained a tumour to blood ratio of 19:1 compared with 2:1 for ¹²⁵I labelled monoclonal antibody against human milk fat globule membrane in mice bearing human breast carcinoma xenografts.⁹ Conventional imaging techniques require a minimum 8:1 ratio for tumour to non-tumour concentration for satisfactory definition of a malignant lesion. Epenetos *et al* were able to identify epithelial cell malignancies with ¹²³I labelled monoclonal antibodies without resorting to subtraction techniques,¹⁰ and similar results may also be achieved using ¹¹¹In as the label.

Another approach to lessen background activity and thus avoid the need for subtraction has been to clear non-tumour bound antitumour antibody by using a second, liposomally entrapped antibody.¹¹ In this instance iodinated polyclonal goat anticarcinoembryonic antigen was the first antibody and a horse antigoat immunoglobulin the second. Accelerated clearance of anticarcinoembryonic antigen was shown in four, and improved gammacamera imaging in three of five patients with gastrointestinal cancer.

Ideally the labelled antibody should recognise an antigen expressed exclusively by tumour cells. The only tumour specific antigen isolated so far in man, however, is the idiotype on the surface immunoglobulin of B cell malignancies¹²: this has not been used for radioimmunolocalisation studies. The expression of tumour antigens is known to be different in

vivo and in vitro,¹³ in metastases and in the primary tumour, and among tumours of the same histological type. For maximum effect, therefore, a cocktail of antibodies recognising a range of different antigenic determinants on heterogeneous malignant cells may have to be used.^{14 15}

Monoclonal antibodies

There are two sorts of antibodies that can be used to identify tumour associated antigens, polyclonal antibodies or the more specific monoclonal antibodies. The largest experience in the radioimmuno-detection of cancer has been with iodinated polyclonal antibodies against tumour associated antigens such as carcinoembryonic antigen, α fetoprotein, and human chorionic gonadotrophin. Inevitably there are cross reactions with those normal tissues that express the antigen—for example, colon in the case of carcinoembryonic antigen. Monoclonal antibodies have many advantages in terms of specificity and purity; they are superior to conventional antibodies for tumour localisation^{16 17} and have been found to target specifically to the cell membrane rather than to antigen in the extracellular space.¹⁶

Specific monoclonals are not likely to be the whole answer to the problems of tumour detection and improved imaging. Unexpected cross reactions may occur between monoclonals and normal tissues, resulting in poor imaging and sometimes dramatic side effects—for example, granulocytopenia.¹⁸ The specific antimelanoma p97 monoclonal has a tumour uptake that is only seven times greater than that of an indifferent antibody in melanoma xenografts in athymic mice (which do not themselves express the antigen), suggesting that there are still many problems with tumour targeting of antibodies.¹⁹ The Charing Cross group found no advantage of monoclonal over polyclonal anticarcinoembryonic antigen antibodies in patients with cancer of the colon and rectum.²⁰

One of the main limitations of the available monoclonal antibodies is that tumour cells are able to modulate a membrane antigen—that is, alter its antigenic expression in the presence of a monoclonal antibody. There is therefore a danger that using an antibody for tumour detection may induce modulation making the tumour resistant should the same antibody later be used for treatment. Further monitoring of the effect of treatment on tumour size using labelled antibodies would also be impeded.

Circulating antigen in the blood will bind with the antibody, forming immune complexes which will interfere with radio-localisation especially over the liver, because of the uptake of the complexes by the reticuloendothelial system and the non-specific binding to hepatocytes and monocytes through the Fc portion of the molecule.^{21 22} Hine *et al* showed that concentrations of circulating carcinoembryonic antigen as high as 1600 $\mu\text{g/l}$ did not prevent detection of tumour.²³ Unfortunately, the circulating plasma concentration of antigen does not indicate which patients have sufficient antigen on the tumour to allow for adequate radioisotope attachment.¹

Certain features of the tumour such as density, vascularity, and size influence the sequestration of antibody. Cystic tumours and those with a loose cellular organisation—for example, dermal tumours—take up antibody more quickly than solid or densely packed tumours.^{10 24} The blood flow and vascular permeability of the tumour affect the delivery of antibody to available binding sites. If the antigen is readily accessible on the cell surface membrane binding will be rapid. Lastly, antibody tends to bind more avidly to viable

tissue than to areas of necrosis and fibrosis^{16 25} and poorly to areas of low grade malignancy,²⁶ leading to false negative results. In a recent study radiolabelled antibodies to carcinoembryonic antigen localised in lymph nodes with no histological evidence of metastatic cancer in nine of 50 patients with a variety of solid tumours.²⁷ The authors postulated that the uptake of antibody was due to retention of carcinoembryonic antigen in lymph nodes draining the tumour, thus giving false positive results. These findings have important clinical implications, since lymphoscintigraphy offers a useful method of determining whether masses found after potentially curative treatment represent residual disease or merely fibrosis.

Antibody molecules are selectively taken up by the tumour and do not localise by simple passive transfer from the serum.^{17 28 29} Pressman has developed a paired labelling technique in which labelled antibodies and control normal immunoglobulin are both injected,²⁹ allowing a distinction to be made between specific localisation of antibodies and the non-specific accumulation of IgG in areas of inflammation or necrosis. When a monoclonal antibody is used to identify the antigen a similar approach using an indifferent antibody of the same immunoglobulin class from the same species but labelled with a different isotope should be used to localise non-specific uptake. Moshakis *et al* were unable to find evidence to support the theory that histiocytic uptake of immune complexes in the tumour contributes to antibody localisation.³⁰ The amount of antibody sequestered by the tumour is only a small fraction of the total, however, and has been shown to vary between 0.1%²¹ and 0.6%¹⁰ of the injected radiolabel.

Radioimmuno-detection would be most useful if it permitted the localisation of clinically undetectable tumours or metastases which are not identifiable with current techniques since this would materially affect the management of the patient. The actual size of the lesion is an important factor for its detection. Two centimetres is the lower limit usually achieved with available gammacameras.² For patients with pulmonary deposits computed tomography gives better resolution, while the uptake of immune complexes in the liver means that radiolabelled antibodies are unsuitable for diagnosing diffuse hepatic spread. Even refinements such as liposomally entrapped second antibody have failed to identify deposits 6 mm diameter.¹¹ When computer emission tomography is used calculations suggest that 1 cm tumours located as deep as 5 cm from the skin could be located with a target to non-target ratio of 5:1.³¹ Mach's group developed a method using single photon emission tomography with radiolabelled monoclonals against carcinoembryonic antigen.³² Sixteen of 17 tumour sites were detected, five of them having a volume of less than 10 cm³. Rectilinear scintigraphy using the same antibodies in some of these patients identified nine of 21 tumour sites, eight of which were larger than 50 cm³. The main limitation of the technique was the failure of the tumour to concentrate sufficient antibody for detection.

Effects on patient

The activity of the dose administered to the patient is in the region of 2 mCi, but has been as high as 4.4 mCi in one report.³ A dose of 2 mCi is in the range delivered with a conventional ¹³¹I thyroid scan. The thyroid needs to be protected with Lugol's iodine or triiodothyronine or both if ¹³¹I is used; protection should start two days before administration of the isotope and continue for at least two weeks after-

wards. The importance of prophylaxis should be emphasised to the patient since otherwise the thyroid may receive unacceptable levels of radiation.⁶ Patients need to be tested for sensitivity to the foreign protein before scanning is performed. In over 400 patients only two instances of a skin reaction to the antibody have been noted. In 50 patients who have received two or three imaging studies no reactions have been observed.¹⁵

Promising work is being done to overcome many of the main difficulties with the sensitivity and specificity of radioimmunodetection. This technique seems likely to be most useful in the detection of occult metastases after the induction of a clinical remission. Radiolabelled antibodies may show lesions at an early stage that are potentially resectable. Improvements may be seen in the future with the use of antibody fragments, F(ab) and F(ab)₂, which have several advantages for use in vivo. They are more rapidly cleared from the blood and do not form immune complexes as readily as whole immunoglobulin, do not bear the antigenic Fc receptors, do

not bind complement or cells with Fc receptors, and do not induce antigenic modulation.

While there are so many technical problems attempts to identify tumour using radiolabelled antibodies remain experimental. Further rigorous objective testing and large scale studies are needed before the method can be advocated for widespread clinical use. Only then—once true tumour specific localisation has been shown in vivo—might targeting of antibodies linked to toxins or drugs be considered.

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